

- (a) preparing a culture of cells which are permissive to the virus and a cell substrate for the production of human vaccines;
- (b) suspending the cells in a culture medium and seeding said cells in said medium at a density of less than 2×10^5 cells/cm² to form a cell culture;
- (c) incubating the cell culture obtained in step (b) at 30 to 40 °C for a period of time between 12 and 144 hours to form an incubated cell culture;
- (d) removing the culture medium from the incubated cell culture of step (c) to form a cell suspension and inoculating the cell suspension with seed virus at a concentration of 0.2 – 0.0001 infectious units per cell to form a second cell culture;
- (e) incubating the second cell culture at 25 to 40° C for a period of time between 12 and 144 hours to form a second incubated cell culture;
- (f) removing the culture medium from said second incubated cell culture, recovering the cells of said second incubated cell culture, washing the recovered cells at least one time to form a collection of washed cells and resuspending the collection of washed cells in a culture medium to form a third cell culture;
- (g) incubating the third cell culture of step (f) at 25 to 40° C for a period of time between 12 and 144 hours to produce a third incubated cell culture which contains cultured virus;
- (h) at least partially harvesting said cultured virus from said third incubated cell culture to form a harvested supernatant, and optionally, adding a stabilizer to said harvested supernatant;

- (i) optionally, repeating step (h) wherein medium removed during said harvesting is replaced and said third incubated cell culture is further incubated for a period of time between 12 and 144 hours after said medium is replaced;
- (j) optionally, removing any cell debris from the harvested supernatant;
- (l) optionally, virally inactivating any non-attenuated virus in said harvested supernatant and;
- (m) storing the virus in said harvested supernatant at a temperature of at most -45°C or lower.

72. (new) The process according to claim 71 wherein the cells of the culture of cells are selected from the group consisting of chicken embryo cells and mammalian cells, said cells being capable of producing interferon when infected by said virus.

73. (new) The process according to claim 72 wherein the cells of the cell culture are selected from the group consisting of chicken embryo fibroblasts, chicken embryo cells, human diploid fibroblasts, monkey kidney cells and fetal Rhesus lung cells.

74. (new) The process according to claim 71 wherein the culture of cells is a primary cell culture.

75. (new) The process according to claim 71 wherein said density is in the range of 1×10^4 - 2×10^5 cells/cm².

76. (new) The process according to claim 75 wherein said density is in the range of 1×10^4 - 1×10^5 cells/cm².

77. (new) The process according to claim 71 wherein said incubating of any of steps (c), (e), (g) and (i) is individually conducted for periods of time between 12 and 72 hours.

78. (new) The process according to claim 71 wherein a stabilizer is added in step (h).

79. (new) The process according to claim 78 wherein the stabilizer is a substance selected from the group consisting of human serum albumin, a peptide, an amino acids, a protein and mixtures of at least two of human serum albumin, a peptide, an amino acid and a protein.

80. (new) The process according to claim 71 wherein the virus is a wild-type virus, an attenuated virus or a recombinant virus.

81. (new) The process according to claim 80 wherein the virus is a Flavivirus.

82. (new) The process according to claim 81 wherein the Flavivirus is Yellow Fever virus.

83. (new) The process according to claim 82 wherein the Flavivirus is an attenuated Yellow Fever virus.

84. (new) The process according to claim 83 wherein the Yellow Fever virus is selected from the group consisting of a YF17D virus strain and a YF17D virus substrain.

85. (new) A process for preparing a human Flavivirus vaccine comprising the steps of:

- (a) preparing a culture of cells which are permissive to Flavivirus and a cell substrate for the production of human vaccines;
- (b) suspending the cells in a culture medium and seeding said cells in a medium at a density of less than 2×10^5 cells/cm² to form a cell culture;
- (c) incubating the cell culture obtained in step (b) at 30 to 40 °C for a period of time between 12 and 144 hours to form an incubated cell culture;
- (d) removing the culture medium from the incubated cell culture of step (c) to form a second cell suspension and inoculating the second cell suspension with

seed Flavivirus at a concentration of 0.2 – 0.0001 infectious units per cell to form a second cell culture;

(e) incubating the second cell culture (d) at 25 to 40° C for a period of time between 12 and 144 hours to form a second incubated cell culture;

(f) removing the culture medium from said second incubated cell culture, recovering the cells of said second incubated cell culture, washing the recovered cells at least one time to form a collection of washed cells, resuspending the collection of washed cells in a culture medium to form a third cell culture;

(g) incubating the third cell culture of step (f) at 25 to 40° C for a period of time between 12 and 144 hours to produce third incubated cell culture which contains cultured virus;

(h) at least partially harvesting said cultured virus from said third incubated cell culture to form a vaccine composition and, optionally, adding a stabilizer to said vaccine composition;

(i) optionally, repeating step (h) to form separate vaccine compositions, wherein medium removed during said harvesting is replaced and said third incubated cell culture is further incubated for a period of time between 12 and 144 hours after said medium is replaced;

(j) optionally, removing any cell debris from the vaccine composition of step (h), or the separate vaccine compositions of step (i), to form an optional further vaccine composition;

(l) optionally, virally inactivating any non-attenuated virus in said vaccine composition, separate vaccine compositions or optional further vaccine composition, to form a virally inactivated vaccine composition;

(m) optionally, lyophilizing the vaccine composition of steps (h), (i), (j) or (l) to obtain a freeze-dried form of the vaccine composition.

86. (new) The process according to claim 85 wherein the cells of the culture of cells are selected from the group consisting of chicken embryo cells and mammalian cells, said cells being capable of producing interferon when infected by said virus.

87. (new) The process according to claim 86 wherein the cells of the cell culture are selected from the group consisting of chicken embryo fibroblasts, chicken embryo cells, human diploid fibroblasts, monkey kidney cells and fetal Rhesus lung cells.

88. (new) The process according to claim 85 wherein the culture of cells is a primary cell culture.

89. (new) The process according to claim 85 wherein said density is in the range of 1×10^4 - 1×10^5 cells/cm².

90. (new) The process according to claim 85 wherein said incubating of any of steps (c), (e), (g) and (i) is conducted for periods of time between 16 and 72 hours.

91. (new) The process according to claim 85 wherein a stabilizer is added in step (h).

92. (new) The process according to claim 91 wherein the stabilizer is a substance selected from the group consisting of human serum albumin, a peptide, an amino acids, a protein and mixtures of at least two of human serum albumin, a peptide, an amino acid and a protein.

93. (new) The process according to claim 85 wherein the virus is a wild-type virus, an attenuated virus or a recombinant virus.

94. (new) The process according to claim 93 wherein the Flavivirus is Yellow Fever virus.

95. (new) The process according to claims 93 wherein the Flavivirus is an attenuated Yellow Fever virus.

96. (new) The process according to claims 94 wherein the Yellow Fever virus is selected from the group consisting of a YF17D virus strain and a YF17D virus substrain.-

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